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MICROBIOLOGICAL FACTORS IN THE MIGRATION OF CERTAIN MINERALS IN SOIL

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16. Abstract

The basic microbiological processes causing mobilization of chemical elements from poorly-soluble compounds are discussed. It is concluded that the most important roles are played by: a) the formation by microorganisms of mineral and organic acids and products forming complex compounds with mineral elements; b) the release of biogenetic alkalis; c) the effect of slimes formed by certain bacteria and algae and d) the life processes of microorgainsms - reducing agents.

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The basic microbiological processes causing mobilization of chemical elements from poorly-soluble compounds are discussed. It is concluded that the most important roles are played by: a) the formation by microorganisms of mineral and organic acids and products forming complex compounds with mineral elements; b) the release of biogenetic alkalis; c) the effect of slimes formed by certain bacteria and algae and d) the life processes of microorganisms — reducing agents. /95*

Life processes of microorganisms form one of the most important factors in the migration of chemical elements in the upper layer of the earth's crust. Great importance was given to this factor by Vernadskiy [4] who considered that the most intense migration of elements in the biosphere was connected with microorganisms.

Acting on insoluble and poorly-soluble chemical compounds, microorganisms convert elements in their composition to a mobile state, thereby raising their migration capacity. In soil these compounds are primarily minerals of soil-forming rock and certain biogenetic secondary formations. The latter include poorly-soluble salts accumulating during the mineralization of plant residue, hydroxides of iron, aluminum and manganese, as well as phytolites — special forms of plant silica.

Quite a bit of material has been accumulated in the literature on the mechanism of the rock-disintegrating effect of soil microflora. On the basis

*Numbers in the margin indicate pagination of original foreign text.

of existing data it can be concluded that, evidently, there is no specialized enzyme apparatus acting directly on the crystal lattices of minerals. Their disintegration, as that of other resistant inorganic compounds in soil, occurs under the influence of metabolic products of microorganisms and slimes, formed by the cells of certain bacteria and algae [5, 13, 15]. Of microbic metabolites the most importance is usually ascribed to organic and mineral acids [2, 11, 12, 15] produced by certain fungi and bacteria. Some of these acids (2-ketogluconic, oxalic, citric and several others), like bacteria slimes, have the ability to form complex compounds with elements in soil minerals. Some authors include among products of microbic origin, playing an important role in weathering processes, phenol compounds, in particular o-diphenols, which are able to form complex compounds with silicon [13].

Depending on the degree of stability of the mineral and the nature of products released by microorganisms, its disintegration can proceed at various intensity and not always lead to the same results. Wagner and Schwartz [15] observed the extremely energetic decay of aluminosilicates under the effect of autotrophic bacteria. Formed by Thiobacillus thiooxidans, sulfuric acid dissolves all elements in the crystal lattice of minerals in proportions corresponding to their ratio in the original rock. Therefore, there was even disintegration of all ingredients of the mineral, which in the course of time would lead to its complete decay. /96

Different results were obtained by these authors with heterotrophic acid-forming and slime-forming microorganisms. In this case elements were always mobilized from aluminosilicates in a certain order, although the absolute amount being dissolved varied. The first to acquire mobility were cations of alkali metals, then alkali-earth metals and then silicon and aluminum in order. Therefore, quantitative ratios between the elements in rock during its weathering under the effect of heterotrophs were changed.

Although the action of microorganisms on rock in the majority of cases was due to chemical reagents formed by them during metabolism, their effect must not be completely reduced to purely chemical weathering, as to a certain degree Müller and Forster tended to do [14]. We must not forget that the microorganism does not relate passively to the interaction between the products of metabolism it releases and minerals. This process holds great physiological importance for it. Under conditions of a medium poor in available minerals, the microorganism uses biochemical reactions in its arsenal, intensely producing the metabolites necessary to extract nutriment from this medium [2]. The intensity of the formation of these kinds of metabolites and their chemical composition depend on the species and the living conditions of the microorganism. The biochemical apparatus which a particular species has available for extracting necessary elements from poorly-soluble compounds is, evidently, to a certain degree "controlled;" this is expressed, for example, in intensification or weakening of slime-formation in bacteria and some algae, intensification or weakening of acid-formation in fungi and bacteria depending on environmental conditions [2] and by the preferential development of bacterial colonies directly on pieces of minerals. "Control" is especially clear in the selective colonization by microorganisms of certain minerals, which Polynov found in his time [7] in lichens and which we detected [1] in fungi and bacteria. Minerals containing vitally important chemical elements were overgrown with microorganisms most intensively and were thereby exposed to biological weathering. This indicates that the action of biogenetic chemical reagents in soil is regulated by living organisms.

The nature and intensity of rock-forming action differ in different species. This appears very clearly when the effect of slime-forming and acid-forming microorganisms on minerals are compared. Extraction of nutrient materials from rock with the help of slimes occurs with direct contact between slime-forming microorganisms and minerals. The effect of strong acid-forming agents is also possible at a distance from the source of necessary elements. This can be illustrated by the results of tests conducted in our laboratories with the effect of Penicillium notatum (a strong acid-forming agent) and /97
Pseudomonas sp. (a slime-forming agent and in part an acid-forming agent) on nepheline — one of the most unstable minerals, a very suitable object for this

kind of comparative study.

Cultures of the above named organisms were grown in two synthetic mediums, differing from each other only by the absence or presence of salts of potassium and sodium. The complete mineral medium had the following composition: K_2HPO_4 — 0.01%, $MgSO_4$ — 0.03%, $CaCl_2$ — 0.01%, NaCl — 0.01%, $(NH_4)_2SO_4$ — 0.1%, glucose — 1%, nepheline — 0.5%. In the incomplete mineral medium, ammonium sulfate was replaced by a corresponding amount of $(NH_4)_2HPO_4$ but salts of potassium and sodium were eliminated.

Both tested microorganisms developed better in the complete mineral medium. In the medium without potassium and sodium, development was delayed and growth was slower. However, by the 12th day after the start of the test we were able to obtain satisfactory growth of both microorganisms and detect clear signs of mineral disintegration.

In comparing the growth of test cultures in various mediums, interesting differences were found between Penicillium notatum and Pseudomonas sp. : Penicillium notatum always formed a film on the surface of the liquid substrate, while Pseudomonas sp. developed evenly throughout the liquid in the complete mineral medium and in the medium without potassium and sodium developed on the bottom of the flask, forming very large slime colonies around particulates of nepheline. The main part of the medium remained almost transparent. In the center of each colony could be seen particles of the

Table 1

Acid formation and disintegration of nepheline by cultures of Penicillium notatum and Pseudomonas sp. in various mediums

Variant	Complete mineral medium				Incomplete mineral medium			
	Content in solution, mg/1			Standard acidity Mg Eq/1	Content in solution, mg/1			Standard acidity Mg Eq/1
	K_2O	Na_2O	Al_2O_3		K_2O	Na_2O	Al_2O_3	
Control	42.0±0.2	55.0±2.0	1.5±0.1	0	4.5±0.1	5.0±0.2	1.9±0.1	0
Penic. notatum	81.0±0.1	116.2±4.2	91.2±2.3	10.2±0.2	15.0±1.0	19.4±1.1	25.2±1.7	10.3±0.2
Pseudomonas sp.	55.5±2.0	74.2±0.8	14.0±1.1	2.0±0.1	0	19.4±1.0	16.6±0.6	1.6±0.1

mineral, especially well marked under low microscope magnification (Fig. 1).

The differences detected in types of growth of the tested organisms could be due to the different character of their effect on the mineral. Determination of standard acidity and the content of potassium, sodium and aluminium cations in the mediums showed that during life processes Penicillium notatum forms large amounts of acid in both mediums (Table 1), causing disintegration of the mineral and dissolution of elements in it in amounts sufficient to support growth of fungus on the surface of the nutrient medium.

In cultures of Pseudomonas sp. standard acidity increased only insignificantly and the extraction of K and Na cations by the formation of acid cannot, evidently, compensate the lack of these elements in the incomplete mineral /98 medium. This is why, developing under conditions of insufficient mineral nutrition, cells of Pseudomonas sp. are established directly on particles of nepheline, affecting the mineral locally, not only as the result of the formation of acid, but also by means of the abundant release of slime.

If, on the basis of data presented in Table 1, we calculate how much K, /99 Na and Al is released from the mineral under the effect of the microorganisms, (i.e., we find the difference between the test and the control) and determine the ratio between dissolved cations in different variants of the test, then the differences in intensity and mechanisms of disintegration of nepheline by the tested microorganisms will become even clearer.

Table 2

Ratio between elements dissolved from minerals in different cultures and in different mediums

Culture	Complete mineral medium					Incomplete mineral medium				
	Dissolved, mg/l					Dissolved, mg/l				
	K_2O	Na_2O	Al_2O_3	$\frac{Al_2O_3}{K_2O}$	$\frac{Al_2O_3}{Na_2O}$	K_2O	Na_2O	Al_2O_3	$\frac{Al_2O_3}{K_2O}$	$\frac{Al_2O_3}{Na_2O}$
Penicil. notatum	39.0	61.2	89.7	2.3	1.5	10.5	14.4	23.3	2.2	1.6
Pseudomonas sp.	13.5	19.2	12.7	0.9	0.6	--	4.4	14.7	--	3.3

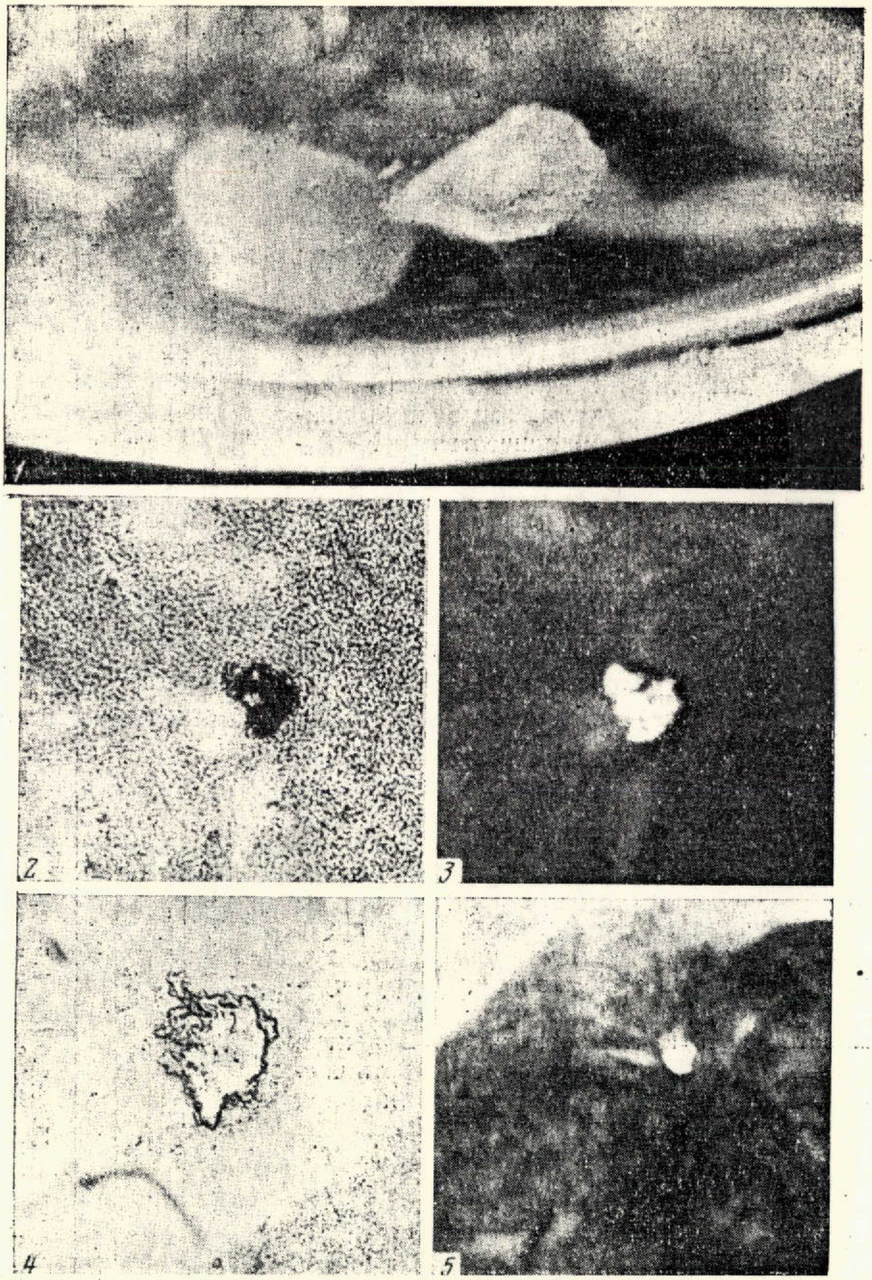

Figure 1. Formation of colonies of Pseudomonas sp. around particles of nepheline (incomplete mineral medium)

1 — colony of bacteria with piece of mineral in center. Mag. 2; 2 — piece of mineral in the center of a bacterial colony. Mag. 350 (20 x 10); 3 — the same, nicol X; 4 — the same with mag. 170 (10 x 10). Easily visible is the edge of the bacterial colony; 5 — initial stage in the formation of a colony around a piece of nepheline. Mag. 350 (20 x 10)

The data given in Table 2 verify that Penicillium notatum has a much stronger effect on the mineral than Pseudomonas sp., and, judging by the growth of the fungus, it is, evidently, on the whole connected with acid formation. Mycelium growing on the surface of the incomplete mineral medium does not come in direct contact with nepheline, but its production of acids is so intense that it causes strong disintegration of the mineral lying on the bottom of the flask and creation of such concentration of necessary elements throughout the medium which will ensure nutrition of the fungus film on the surface. The ratios between elements entering the medium as the result of the disintegration of nepheline are practically the same in the complete and incomplete mineral mediums in cultures of Penicillium notatum.

The effect of Pseudomonas sp. on the mineral is much less intense than that of Penicillium notatum and in part, probably, this is why it is strictly local in the medium not containing K or Na.

In cultures of Pseudomonas sp. ratios between cations in the solution released in the disintegration of nepheline are in conversion from complete mineral nutrition to the incomplete mineral medium sharply altered. A significant part of the released elements are used by the cells and only the excess, not needed by the organism, is dissolved.

Judging by the amount of aluminum accumulated in the solution, the degree of disintegration of the mineral under the effect of life processes of Pseudomonas sp. is approximately the same in both mediums and even slightly higher in that deprived of K and Na. Nevertheless, dissolved K is found only in the complete mineral medium and the ratios between Na_2O and Al_2O_3 in this medium were much narrower than in the medium lacking K and Na. Therefore, all released K and a large part of Na are used by living cells; Al enters the medium in relatively large amounts and accumulates there.

Thus, the bacterial film which covers the mineral acts as a unique living filter, having a selective capacity in relation to certain chemical elements. The abundant slime formation in the incomplete mineral medium indicates that

the main means of extracting nutrient materials from minerals by this kind of bacteria is not only the release of acids, but also of slimes (chemical reaction between the slime and elements in the rock). It is possible that as the result of this reaction some kind of complex compound is formed, as it is /100
known that bacterial slimes are able to form such compounds with components of crystal lattices of minerals [10].

The release by microorganisms of metabolic products having the ability to complex are, evidently, widespread in nature and play an important role in soil formation. In particular, it is very important for the mobilization of Fe from soil minerals and other poorly-soluble forms of this element. In combination with reducing reactions caused by life processes of certain bacteria and leading to the reduction of ferric oxide to ferrous [6], this is one of the most important biogenetic factors in the migration of this element.

Ratios between different reagents of microbic origin, due to the mobility of iron, can be illustrated by the data we obtained in studying the disintegration of a number of ferriferous minerals under semi-anaerobic conditions.

Tests were conducted with cultures of Clostridium pasterianum and stock cultures of pseudomonads, obtained from soil, which have a clearly pronounced ability to reduce ferric oxide. The mixture of cultures was sown in a modified Bromfield medium [9] in which sucrose was replaced by glucose and iron hydroxide by corresponding minerals. Minerals were first crushed until particles measuring 0.25 mm were produced. To create relative anaerobiosis, the mediums were poured into flasks in a thick layer, almost up to the very corks. Tests lasted a month; several times during this period chemical analysis was made of the medium. In cultural liquid were determined standard acidity, content of ion forms of iron (ferric oxide by the colorimetric method with sulfosalicylic acid, ferrous oxide by colorimetry with α-α-dipyridyl) and iron in organic mineral complex compounds (calculated by the difference between the total content of all forms of iron, determined in preliminary ashing tests, and the content of its ion forms).

The development of bacteria was always accompanied by an increase in standard acidity in the medium and the dissolution of iron.

The role of organic complexing agents (resulting from the conversion of glucose by microorganisms) in the disintegration of minerals is especially clear in tests with limonite and marcasite.

Although the first of these minerals contains only trivalent iron, during nearly the entire observation period ion forms of ferric oxide in solution were almost never observed. As evident from the data given in Fig. 2, it appeared in trace amounts only in old cultures. During the first two weeks a gradual increase was ascertained in standard acidity and the accumulation in the medium of bound non-ion, as well as ion bivalent iron; first iron of complex compounds predominated and later, ferrous oxide. Evidently, organic acids formed during life processes of bacteria, acting on limonite, immediately form complex compounds with iron, which are then partially disintegrated and the released iron, under the effect of bacterial dehydrogenase [8], converts to a ferrous state. The possibility of the reduction of trivalent iron at the same time as complexing is not excluded.

As active life processes of bacteria fade away, reducing processes weaken and a small amount of trivalent iron is accumulated. Attracting attention is the sharp reduction in the content of all forms of dissolved iron in the cultural medium by the end of the test.

All these processes reached an incomparably wide scope in mediums with /101 marcasite — a mineral containing iron in bivalent form. Here was found unusually rapid and intense dissolution of the element. The content of bivalent and complex iron in the medium increased at approximately the same rate and reached significantly higher levels than in tests with limonite. As seen from the accompanying graph (Fig. 2), as the culture aged, oxidation processes in ferrous oxide developed. Probably, as life processes of bacteria abated in old cultures, processes which are purely chemical, no longer controlled by microorganisms, come into effect and in the upper layer of the liquid the degree of anaerobiosis was not sufficient to preserve iron in bivalent form. As in an analogous test with limonite, by the end of the observation period the total iron content in solution was reduced, but in this case, significantly more sharply (Fig. 2, II).

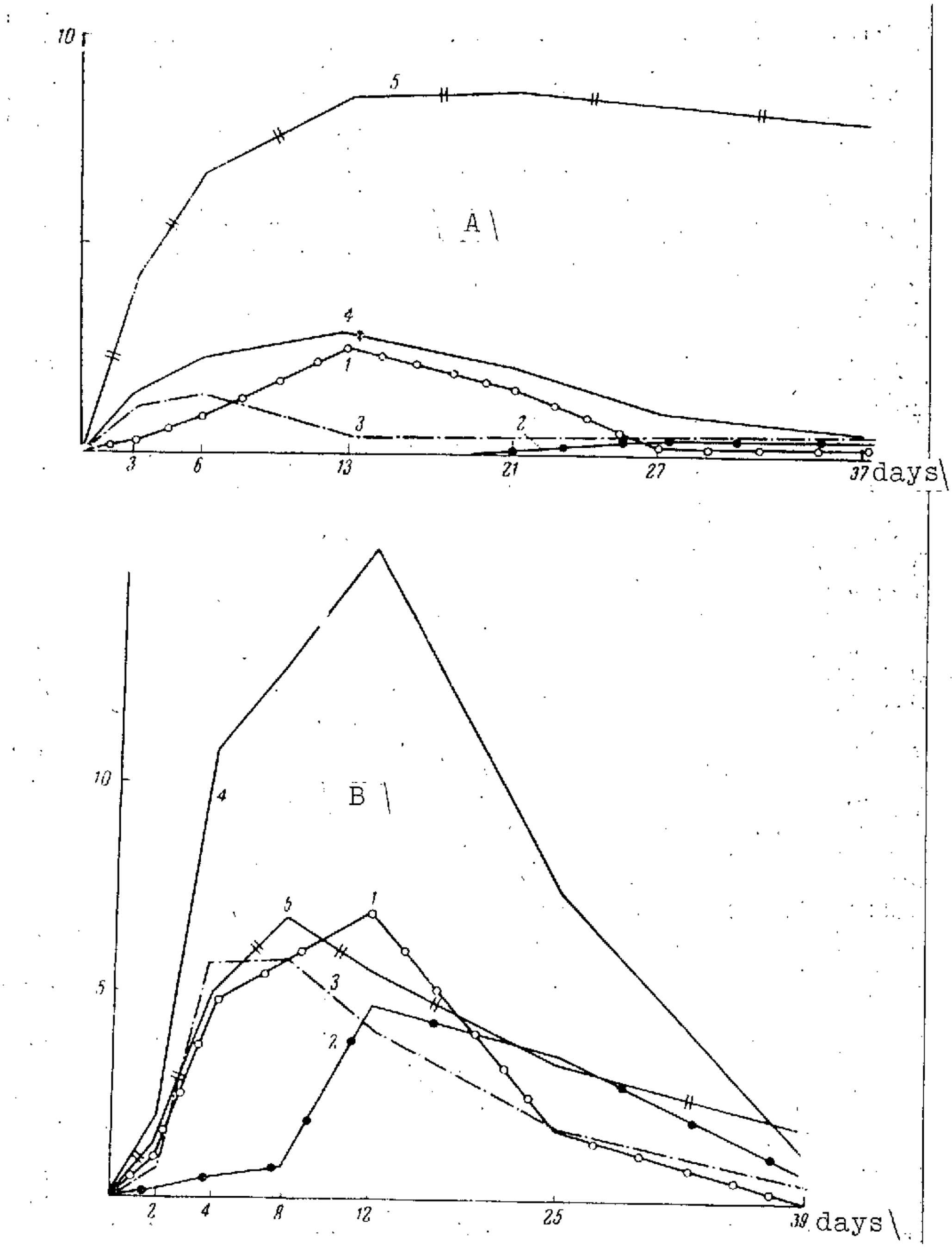

Figure 2. Dynamics of the conversion of forms of iron (mg/100 ml of medium) under the effect of microorganisms to limonite (I) and marcasite (II).

1 — ferrous oxide; 2 — ferric oxide; 3 — non-ionic organic iron (complex); 4 — total iron; 5 — standard acidity (vertically, in ml of 0.01 n acid per 100 ml of medium)

Reduction of the contents of iron in solution can have only one explanation: obviously, precipitation of iron from solution is taking place in the form of some newly-formed insoluble compounds. It appears most probable that these compounds under given conditions are ferroorganic compounds and iron hydroxide. In favor of the first possibility speaks the fact that in old cultures with marcasite we have simultaneous reduction not only of the amount of dissolved iron, but also of titrated acidity (Fig. 2, II).

The possibility of the precipitation of hydroxide of iron is determined by the presence of trivalent ion forms of this element in solution and the pH of the medium, which in none of the variants of the test fell below 5.0.

In the mobilization of iron from minerals the predominant role is, evidently, played by two biogenetic factors: the formation of microorganisms during their metabolism of organic complexing agents of an acid nature and the life processes of iron-reducing bacteria. If in the first case release of iron from crystal lattices of minerals is provided, in the second iron is converted from the less mobile ferric oxide state to the more mobile ferrous. As is known, bivalent iron can be found in solution under conditions which do not permit mobility of the trivalent form of this element.

Besides microbic slimes released by microorganisms of mineral and organic acids and various products having the ability to complex, as well as reducing reactions by bacteria, an important role in the disintegration of minerals is played by microbic metabolites of an alkaline nature.

Studies we made [3] showed that some poorly soluble compounds are disintegrated primarily by microorganisms which are acid forming agents (aluminosilicates) and others by microorganisms which are alkali-forming agents (quartz, phytolites). Thus, biogenetic alkali-formation is an important factor in the mobility of certain chemical elements, particularly silicon.

Let us dwell a little longer on one aspect of this problem.

As it is known that with the disintegration of aluminosilicates under the effect of heterotrophic acid-forming agents and slime-forming agents not all

elements in the crystal lattice are released at the same rate [15], significant changes can be expected in the chemical composition of the original minerals, in particular a gradual transformation of primary minerals into secondary. The character of changes can to a certain degree be evaluated by comparing quantitative ratios between individual elements released by the disintegration of the mineral under the effect of microorganisms with their ratio in the original aluminosilicate. Let us consider our data, obtained in /102
model tests with nepheline. Although this mineral is very unstable under natural conditions and, probably, this is why it is rarely found in the soil, it is very suitable for studying the biological mechanism of weathering, as appreciable results can be obtained in a relatively short period of time. It can also be assumed that the general regularities found in tests with nepheline can also be transferred to other objects. The figures given in Table 3 charac- /103
terize ratios between elements contained in the original nepheline and those same elements dissolved from nepheline in cultures of various age.

Table 3

Ratio of elements in original nepheline and in cultural liquid

Variant	SiO_2	Al_2O_3	Na_2O	K_2O
Original nepheline	1	0.8	0.3	0.1
Penicillium notatum:				
10 days	1	0.9	0.6	0.2
20 days	1	1.6	0.8	0.5
30 days	1	3.8	1.1	0.8
Pseudomonas sp.:				
10 days	1	0.6	0.5	0.2
20 days	1	0.7	0.4	0.2
30 days	1	0.9	0.5	0.3

The most significant changes are found in cultures of Penicillium notatum, where the ratio of K_2O/SiO_2 increases by the end of the observation period 8 times in comparison with that between these oxides in the original mineral, Na_2O/SiO_2 increases almost 4 times and Al_2O_3/SiO_2 almost 5 times. Therefore, in the remainder of nepheline the reverse process occurs: the relative content of SiO_2 increases and the K_2O content drops very sharply.

Pseudomonas sp. has a weaker effect on the mineral, but in this case certain changes are also detected in its content.

It can also be assumed that other, more stable aluminosilicates should, under the effect of microorganisms, undergo analogous transformations; however, detection of these changes requires longer tests.

Conclusions

1. Life processes of microorganisms, responsible for the mobility of chemical elements, form the most important factor in their migration in soil. Elements are mobilized from poorly-soluble natural compounds primarily under the effect of metabolic products released by microorganisms.

2. The most important microbiological factors of the mobility of mineral elements are:

a) the formation by microorganisms of mineral and organic acids and products, forming complex compounds with certain elements;

b) the release by microorganisms of biogenetic alkalis;

c) the effect of slimes formed by certain bacteria and algae;

d) the life process of microorganisms, having the ability to cause reduction processes.

3. Aluminosilicates are most intensively disintegrated under the effect of microorganisms which are acid-forming agents and complexing agents. Quartz and phytolites are disintegrated under the effect of alkali-forming microorganisms. In the mobilization of iron an important role is played by iron-reducing bacteria.

4. The release by microorganisms of chemical reagents which mobilize elements from poorly-soluble compounds has an important ecological-physiological meaning for organisms and is a process controlled by biological factors. Its intensity depends on the species of the microorganism and the conditions of the medium in which it develops. Of great importance is the content of easily-accessible forms of necessary elements in the medium.

5. When minerals are disintegrated under the effect of heterotrophic bacteria, elements are not mobilized in proportion to their content in the original material and, therefore, the chemical composition of the mineral gradually changes. / 104

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